under 37 C.F.R. § 1.136(a), and any fees required therefore (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Specification:

In the section "Brief Description of the Drawings", please delete the section beginning at page 17, line 10 and ending at page 18, line 14.

Please substitute the following paragraph for the paragraph beginning on page 128, line 10:

Following addition of CNTF, a receptor complex forms that consists of CNTF, CNTFRα, gp130, and LIFRβ. Immunoprecipitation (IP) of the receptor complex with antibodies against LIFRβ or gp130 (not shown) following cell lysis in the detergent Brij 96 results in the co-purification of a 130 kDa protein that is tyrosine phosphorylated. LIF and OSM, which also bind to and heterodimerize gp130 and LIFRβ (Gearing *et al.*, *Science 260*:1434-1437 (1992); Baumann *et al.*, *J. Biol. Chem. 268*:8414-8417 (1993); Davis *et al.*, *Science 250*:1805-1808 (1993)), also show association and tyrosine phosphorylation of a protein with an identical appearance. The purified receptor complex also shows associated protein tyrosine kinase activity *in vitro* giving rise to tyrosine phosphorylation of both gp130 and LIFRβ, as well as the associated 130 kDa protein. Tyrosine kinase activity is also associated with LIFRβ in the absence of CNTF, although the 130 kDa protein is either not present or not significantly phosphorylated in

the absence of the factor. Other experiments showing that this *in vitro* kinase activity has the same sensitivity to staurosporine as that observed upon addition of CNTF to intact cells suggested that this associated tyrosine kinase activity is relevant to that which is required in the cell to mediate CNTF-induced responses. Furthermore, the 130 kDa protein appears to be a good candidate for this kinase since lysis of the cells in NP-40 does not give co-purification of either the 130 kDa protein or tyrosine kinase activity (not shown).

C 1 CONT.

Please substitute the following paragraph for the paragraph beginning on page 129, line 6:

C 2

Experiments using specific antisera raised against portions of Jak1, Jak2, or Tyk2 reveal that all 3 of these kinases can become tyrosine phosphorylated following stimulation by CNTF, LIF, OSM, and IL6. CNTF induces tyrosine phosphorylation of both Jak1 and Jak2 in EW1 cells, and these proteins appear to co-migrate with 130 and 131 kDa proteins that co-purify with the receptor complex immunoprecipitated with a-LIFRβ. Furthermore, the addition of IL6 + sIL6Rα, as well as LIF and OSM (not shown) to EW-1 calls also results in phosphorylation of Jak1 and Jak2 but not Tyk2. In contrast, IL6 stimulated U266 cells give tyrosine phosphorylation of Tyk2 and Jak1 without apparent change in the phosphorylation status of Jak2. OSM treated SK-MES cells reveal tyrosine phosphorylation of primarily Jak2, with smaller changes in Tyk2 and Jak1. In each of these cases, tyrosine phosphorylation of the Jaks or Tyk2 is associated with an increase in their in vitro tyrosine kinase activity (not shown). These results stand

in contrast to previous results showing that stimulation with GM-CSF, EPO, G-CSF, IFN-γ, or IL-3 only result in tyrosine phosphorylation of Jak2 ((Argetsinger *et al.*, *Cell 74*:237-244 (1993); Silvennoinen *et al.*, *Proc. Natl. Acad. Sci. USA* (in press;1993); Witthuhn *et al.*, *Cell 74*:227-236 (1993)). We conclude from these experiments that the CNTF family of factors can activate Jak1, Jak2, and Tyk2, although there is some variability in which Jak/Tyk family member is activated in a particular cell.

C2 Cont.

Please substitute the following paragraph for the paragraph beginning on page 130, line 2:

Transient transfections in COS cells were used to determine whether the Jaks could associate with the β receptor components in the absence of factors. These experiments used carboxyl terminally epitope-tagged versions of LIFRβ containing the 10 amino acid portion of c-myc that is recognized by the monoclonal antibody 9E10 (Davis *et al.*, *Science 253*:59-63 (1991)). COS cells were co-transfected with appropriate expression vectors encoding full length versions of LIFRβ and Jak1 or Jak2, and Brij 96 lysates were immunoprecipitated with 9E10 and then blotted with the antisera against either Jak1 or Jak2. These experiments show that either Jak can associate with LIFRβ in the absence of any added ligand. Furthermore, a truncated version of LIFRβ which retains only the first 76 amino acids of the cytoplasmic domain is fully capable of binding to Jak1 and Jak2 as well. This implicates the membrane proximal region of LIFRβ as the Jak binding domain, which is consistent with the homology between this region of the receptor with those in gp130 and EPOR that have been shown to be

C3

C3
CON't

required for signal transduction upon factor binding (Murakami *et al.*, *Science 260*:11349-11353 (1991); Witthuhn *et al.*, *Cell 74*:227-236 (1993)).

Please substitute the following paragraph for the paragraph beginning on page 130, line 21:

C4

Further experiments in COS cells were undertaken to establish whether co-transfection of the receptor β-components with the Jaks could reconstruct a ligand-induced functional response. Epitope-tagged gp130FLAG and IL6 were chosen for these experiments, since gp130 homodimerizes and becomes tyrosine phosphorylated in response to IL6 + soluble IL6Rα, obviating the need for co-transfection with LIFRβ (Murakami *et al.*, *Proc. Natl. Acad. Sci. USA 88*:11349-11353 (1993); Davis *et al.*, *Science 260*:1805-1808 (1993)). Following stimulation with IL6 + sIL6Rα, neither mock transfected nor gp130FLAG transfected COS cells revealed substantial tyrosine phosphorylation of gp130 following immunoprecipitation with anti-FLAG and α-PTyr immunoblotting. In contrast, co-transfection with either Jak1, Jak2, or both Jak1 and Jak2 gives rise to a substantial increase in the induced tyrosine phosphorylation of gp130 upon stimulation with IL6 + sIL6Rα.

In the Claims:

Please substitute the following claim 43 for the pending claim 43: